USING RECOMBINANT DNA TECHNOLOGY TO STUDY THE BEHAVIOR OF RETROVIRUSES. H.E. Varmus. Department of Microbiology and Immunology, University of California, San Francisco, CA 94143.

Recombinant DNA technology is now central to our efforts to understand the complex genetic behavior of retroviruses. I will discuss several areas of recent research which illustrates the powers of this methodology to explore gene regulation and oncogenesis by these agents.

(1) Structure of proviral DNA. We have cloned and sequenced DNA fragments containing host-viral junctions from cells bearing integrated DNA of Rous sarcoma virus (RSV) (1) or mouse mammary tumor virus (MMTV) (2). Proviruses appear to be structurally similar to transposable elements of bacteria, yeast, and Drosophila. An internal region, coding for one or a few genes, is flanked by long terminal direct repeats (LTRs) which conclude with short inverted repeats; the entire provirus is flanked by a short direct repeat generated by duplication of a cellular sequence present once at the integration site. Integration can occur at many sites, perhaps at random, in the host genome, but specific sites in viral DNA, two nucleotides from the predicted ends of unintegrated linear DNA, are invariably joined to cellular DNA.

(2) Insertional mutagenesis. Since proviruses can enter many sites in host genomes, they should act as mutagens by insertion within genetic units. We have tested this presumption by using murine leukemia virus (MLV), a nontransforming retrovirus, as a mutagen in rat cells transformed by a single RSV provirus (3). Two insertion mutants were isolated as morphological revertants, both bearing MLV proviruses in a region of the RSV provirus which lies between the probable promoter of RNA synthesis in the LTR and the gene (src) responsible for transformation. In this position, the MLV proviruses interfere with the synthesis and/or processing of RSV src mRNA.

(3) Proviral excision. In one of the two insertion mutants described in the preceding paragraph, the MLV proviral "mutagen" is excised at low frequency by recombination between its LTR's, reactivating expression of src and leaving behind one copy of an LTR (3).

(4) Integration as a determinant of oncogenesis. Bursal (B-cell) lymphomas induced in chickens by avian leukosis virus (ALV), a virus lacking a transforming gene, frequently contain a single new ALV provirus with structural defects preventing expression of viral genes (4). However, the ALV proviruses in different tumors are

found in a common region (4,5) shown to include the cellular progenitor (c-myc) of the transforming gene of the myelocytomatosis-29 virus (v-myc) (6). The insertion of ALV DNA in the vicinity of c-myc enhances the expression of this gene, either by the "promoter insertion" mechanism favored by Hayward et al (5,6) or other mechanism suggested by proviruses positioned on the 3' side of c-myc or in the opposite transcriptional orientation on the 5' side of c-myc (7). Tumors induced by the mouse mammary tumor virus (MMTV), another retrovirus lacking a transforming gene, also exhibit proviruses at a preferred site, but no oncogene has yet been identified at that site.

(5) Retroviruses as transducing agents. The transforming genes of RNA tumor viruses are closely related to normal cellular genes believed to be their progenitors (8). We have isolated chicken cell DNA encompassing c-src from a library of recombinant lambda phage and found the gene to be interrupted by sequences, presumably introns, not present in viral src (9).

(6) Retroviral genes and the biochemical basis of mutation. We have isolated and characterized over thirty src mutants in a rat cell line transformed by a single RSV provirus (10). Wild type, mutant, and back mutant genes have been cloned in procaryotic vectors to establish the sequence alteration which affect the phenotype in a few interesting cases; in one case, a +1 frameshift mutation reveals a second functional AUG within src.

(7) Retroviruses as eukaryotic vectors. The LTRs of retroviruses appear to regulate and initiate transcription of viral genes; hence linkage of genes to LTRs in vitro may expedite expression in eukaryotic cells. We have linked LTRs of RSV and MMTV to the thymidine kinase (tk) gene of herpes simplex virus and introduced them into tk- cells by microinjection or DNA transformation. In this way, we have demonstrated the promoter function of LTRs, defined the region of the MMTV LTR which responds to steroid hormones, and identified an LTR function which enhances expression from linked heterologous promoters. We found no evidence, however, of preferential integration at sequences within the LTR under these experimental conditions.

References

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